Local Response to Cardiac Overload on Myosin Heavy Chain Gene Expression and Isozyme Transition

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It is uncertain whether the shift of cardiac myosin heavy chain (MHC) during pressure overload can be induced by some intrinsic factors or by the stress imposed directly on the individual myocytes. To study whether the changes in cardiac MHC gene expression produced by one-sided overload are limited to the involved ventricle or extend to the other ventricle, we examined MHC gene expression and isozyme transition in the left and right ventricles in aortic coarctated and pulmonary artery-banded rats. It has been confirmed that the pressure overload is indeed limited to the loaded ventricle. The results showed that, compared with sham-operated rats, there was no significant induction of the β-MHC messenger RNA and corresponding protein in the unloaded ventricle, whereas significant induction was observed in the overloaded ventricle. These results demonstrated that the changes in MHC gene expression and isozyme produced by one-sided ventricular overload are limited to the involved ventricle. We conclude that the MHC gene regulation during hemodynamic overload may not be induced by intrinsic factors, such as hormones, catecholamine, or atrial natriuretic peptide, but is induced by direct local response to increased load. (Circulation Research 1990;66:1067–1073)

Changes in the relative amounts of three cardiac myosin heavy chain (MHC) isozymes (V1, αα-homodimer; V2, αβ-heterodimer; and V3, ββ-homodimer) are believed to be responsible in part for altered cardiac performance during hypertrophy.2–4 Alpert et al5–7 showed that a high proportion of V3 isozyme (dominant during pressure overload) is associated with relatively slow, economical tension development during isometric contraction, whereas a high proportion of V1 isozyme (dominant during thyrotoxicosis) is associated with a faster, but less economical, force development.

Hemodynamic overload, either pressure- or volume-induced on rat and rabbit ventricles8–10 and human atria,11–13 has been shown to induce the α- to β-MHC isoform transition, and this process is regulated by pretranslational mechanisms.14–18 Furthermore, various hormones, including thyroid hormone,18–23 insulin,24 glucocorticoids,25 sex steroids,26 and growth hormone,27 can modulate the phenotypic expression of the α- and β-MHCs. Recently, sequences in the 5'-flanking regions of several thyroid hormone-sensitive genes were analyzed and found to be important for thyroid hormone regulation.28–30 However, it was clearly recognized that the shift of the MHC during pressure overload occurs without any relation to the serum thyroid hormone levels (authors’ unpublished observation). Therefore, it is uncertain whether the shift of MHC during pressure overload can be induced by other intrinsic factors or by the stress imposed directly on the individual myocytes. Cooper et al31 demonstrated that the increased load itself is the primary cause of cardiac hypertrophy in response to a hemodynamic overload and that catecholamines are not mediators of this response. In contrast, some previous studies32 on cardiac MHC transition during pressure overload showed that changes in cardiac MHC occurred in the right ventricle of aortic coarctated animals. However, no definite conclusion as to whether these changes were produced by the left ventricular pressure overload can be made from these studies, because a detailed hemodynamic study was not performed.

In the present study, we examined whether the changes in cardiac MHC gene expression and
isoenzyme produced by one-sided ventricular overload are limited to the involved ventricle or extend to the other one, using the rat model in which it has been confirmed that the pressure overload is indeed limited to the loaded ventricle.

Materials and Methods

Animals and Surgical Procedures

Ten-week-old male Wistar Imamichi rats, weighing approximately 300 g at the time of surgery, were used. Left ventricular overload was induced by coarctation of the aorta, which was produced by knotting a thread around the abdominal artery just above the renal artery with a 20-gauge needle. Right ventricular overload was induced by main pulmonary artery banding (outer circumference, 5 mm). These operations were performed under pentobarbital anesthesia (15 mg/300 g body wt i.p.). For these studies, aortic coarctated (CoA) rats, pulmonary artery–banded (PAB) rats, and, as controls, sham-operated (Sham) rats for the CoA and the PAB rats were prepared. At 1, 2, 3, and 4 weeks after operation, animals were weighed and their blood pressure was measured. Blood samples were taken, and the rats were killed. The heart was rapidly excised, the left and right ventricles were separated, and the free wall thickness and weight were measured. The ventricles were immediately frozen in liquid nitrogen, stored at −80°C, and subjected to MHC messenger RNA (mRNA) and isoenzyme analyses.

MHC mRNA Analysis (S1-Nuclease Mapping Analysis)

Total cellular RNA was isolated from the myocardium by the hot phenol procedure and was stored at −20°C in ethanol. The DNA probe used in this study was the 3' end Pst I fragment of pCMHC mini5 (β-type MHC complementary DNA clone) provided by Dr. B. Nadal-Ginard, Harvard Medical School, Boston, Massachusetts. RNA-DNA hybridization was carried out in DNA probe excess to 30 µg of the total RNA for 18 hours at 42°C in 80% formamide. S1-nuclease digestion was performed for 1 hour at 25°C, and the digestion products were run on 6% polyacrylamide/8.3 M urea sequencing gel. The proportions of the α- and β-MHC mRNA were quantitated by measurement of the x-ray film density exposed from the gel by a laser densitometer (Ultrascans XL, Pharmacia LKB, Uppsala, Sweden) and were calculated by Gaussian curve.

MHC Isozyme Analysis

The myosin was extracted from the myocardium at 4°C with Hasselbach-Schneider solution (final concentration: 0.6 M KCl, 0.1 M potassium phosphate, 10 mM sodium pyrophosphate, and 1 mM MgCl₂, pH 6.4). The isoforms were separated by 3.7% polyacrylamide gel electrophoresis in the presence of 20 mM sodium pyrophosphate, according to the modified method of Hoh et al. The density of each band (V₁, V₂, and V₃) was quantitated by laser densitometer. The proportion of each band was calculated by the area of the resolved Gaussian curves, and the half value of V₂ density was added to the values of V₁ and V₃.

Blood Pressure

Blood pressure was monitored under pentobarbital anesthesia (15 mg/300 g body wt i.p.) before the animals were killed. A heparinized saline–filled Teflon catheter was inserted into the right carotid artery or right jugular vein. The left and right ventricular peak-systolic pressures (LVSP and RVSP) and end-diastolic pressures (LVEDP and RVEDP) were measured by a physiological pressure transducer (model P10EZ, Statham-Gould Instruments, Cleveland, Ohio).

Left and Right Ventricular Free Wall Thickness and Weight

Left and right ventricular free wall thickness (outflow portion) and right ventricular weight were measured immediately after the ventricles were separated. To express the value of the left and right ventricular free wall thickness, the thickness (in millimeters) was normalized to the body weight (in milligrams). The ventricular weight (in milligrams) was also normalized to the body weight (in grams).

Levels of Thyroid Hormone in the Serum

Serum was separated immediately after blood sampling and was stored at −20°C. Serum concentrations of total thyroxine and triiodothyronine were measured by radioimmunoassay (Clinical Assays, Baxter Healthcare Corp, Cambridge, Massachusetts).

Statistics

The results were expressed as mean±SD. Statistical comparisons between the pressure-overloaded group and the respective Sham group were carried out by use of analysis of variance and unpaired Student’s t test. If the significance level of the F test was less than 0.1, we rejected the hypothesis that the two variances were equal and used an approximate t test (Aspin-Welch method). The relative data were evaluated after transformation to normality by the arcsine transformation. A value of p<0.05 was considered significant.

Results

Blood Pressures

The LVSP in the CoA rats and the RVSP in the PAB rats were significantly higher than those in the Sham rats, whereas the RVSP in the CoA rats and the LVSP in the PAB rats showed no significant increases compared with the Sham rats (Figure 1). LVEDP and RVEDP in the CoA and PAB rats showed no significant increases compared with the Sham rats. These results demonstrate that in these animal models, the
pressure overload was limited to the loaded ventricle and did not extend to the other side.

**Left and Right Ventricular Free Wall Thickness and Weight**

Significant increases in overloaded ventricular free wall thickness were recognized in both the CoA and PAB rats compared with the Sham rats, whereas in the other ventricle, there were no significant increases in free wall thickness in either the CoA or PAB rats compared with the Sham rats (Figures 2A and 2B). To elucidate more clearly the right ventricular hypertrophy in the PAB rats, the right and left ventricular weights in the PAB rats were measured...
Changes in MHC Gene Expression and Isozyme Transition by One-sided Ventricular Overload Could Be Recognized Only in the Involved Ventricle

Total cellular RNA and crude myosin protein were extracted from the left and right ventricles of the same animals. The proportion of the mRNAs coding for the α- and β-MHCs were quantitated by S1-nuclease mapping analysis. Figure 3 shows the changes in the relative level of β-MHC mRNA after operation. In the CoA rats, there was a significant accumulation of the β-MHC mRNA in the left ventricle (overloaded ventricle, 30–39%) compared with the Sham rats (18–21%), whereas in the right ventricle (unloaded ventricle), no differences in the β-MHC mRNA levels between the CoA (13–20%) and Sham (12–15%) rats were observed. Also, in the PAB rats, there was a significant accumulation of the β-MHC mRNA in the right ventricle (overloaded ventricle, 29–37%) compared with the Sham rats (13–19%), but in the left ventricle (unloaded ventricle), there was no significant accumulation (19–35%) compared with the Sham rats (20–34%).

The changes in the relative level of V3-MHC isozyme (Figure 4) showed nearly the same tendency as those at the gene level. In the CoA rats, there were significant increases in the V3-MHC isozyme in the left ventricle (overloaded ventricle, 27–35%) compared with the Sham rats (13–18%). In the unloaded right ventricle, however, no significant differences between the CoA (12–19%) and Sham (10–17%) rats were observed. Also, in the PAB rats, there was a significant accumulation of the V3-MHC isozyme in the overloaded right ventricle (29–36%) compared with the Sham rats (13–18%), but in the unloaded left ventricle, there was no significant increase (20–34%) compared with the Sham rats (18–32%).

These results demonstrate that the changes in MHC gene expression and isozyme produced by one-sided ventricular overload are limited to the involved ventricle and do not extend to the other one.

Serum Concentrations of Thyroid Hormone

Circulating total thyroxine and triiodothyronine levels were measured to examine whether the induction of β-MHC gene during pressure overload may possibly be associated with a decrease in thyroid hormone levels. As shown in Figure 5, there were no significant differences in either thyroxine or triodo-
thyroxine levels between the CoA and Sham rats or the PAB and Sham rats.

Discussion

Either pressure or volume overload can induce the α- to β-MHC isoform transition, and this process is regulated by pretranslational mechanisms. It has been shown that the α- to β-MHC shift during pressure overload has no relation to the serum thyroid hormone levels (authors' unpublished observation). This finding was also confirmed in the present study, and this result shows that cardiac MHC gene regulation by pressure overload and by thyroid hormone is mediated by different factors.

The results presented here demonstrate that the changes in MHC gene expression and isozyme produced by one-sided ventricular overload are limited to the involved ventricle and do not extend to the other one. These results are not in agreement with some previous findings on the effect of aortic coarctation. The time courses of the relative amounts of the V3-myosin heavy chain (MHC) isozyme. Left panel shows the changes in relative amounts of the V3-MHC isozyme in the left ventricle (LV) and right ventricle (RV) in the aortic coarctated (CoA) rats. Right panel shows changes in the RV and LV in the pulmonary artery-banded (PAB) rats. The changes in the relative level of V3-MHC isozyme tended to be almost the same as those at the gene level. Preoperative normal data. Number of animals are in parentheses. Values are mean±SD.

![Figure 4](image1)

![Figure 5](image2)
tation to right ventricle on MHC isoyme transition. Izumo et al. demonstrated that the right ventricle can undergo a degree of MHC isoyme transition similar to that seen in the left ventricle in aortic coarcted animals. However, they used only three animals, which is too few to make this conclusion. Martin et al. demonstrated a small but marginally significant (p < 0.05) increase in the percent V3 isoyme in the right ventricle in adult animals with aortic coarctation compared with corresponding sham-operated control animals. Their procedure for producing coarctation of the aorta was the same as ours, but they did not measure the blood pressure and began their study 5 weeks after surgery. Lacks et al. demonstrated that left ventricular cells in dogs with pulmonary arterial banding were significantly longer than in normal hearts, although this increase was less than that observed in the right ventricle. They suggested that a substance that can produce hypertrophy may be released from the overloaded right ventricle and transported to the left ventricle. However, they used only five mongrel dogs weighing 16–22 kg. Right ventricular pressure overload was induced by banding the main pulmonary artery, and systolic pressure of 50 mm Hg or greater was produced. They killed the dogs from 17 to 48 weeks after surgery, and the data were not compared with sham-operated controls, but with normal controls that were reported in another of their studies.

Since the hemodynamic studies were not performed on a sufficient number of experimental animals and sham-operated animals to clarify whether the overload was limited to the loaded ventricle or extended to the opposite one, no definite conclusion can be made from these studies.

In our study, animal models clearly confirmed that pressure overload was limited to the loaded-side ventricle. Using these animal models, we could further confirm that one-sided ventricular overload had no effect on the shift of MHC in the opposite ventricle.

From these results, we conclude that MHC gene regulation during hemodynamic overload may not be induced by intrinsic factors, such as hormones, catecholamine, or atrial natriuretic polypeptide, but by a direct local response to increased load. An important future study will be to elucidate the precise molecular mechanisms of MHC gene regulation by hemodynamic overload.

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